PROCEEDINGS

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OF

NEW SOUTH WALES.

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ON A NEW SPECIES OF ENTEROPNEUSTA (PTYCHO-DERA AUSTRALIENSIS) FROM THE COAST OF NEW SOUTH WALES.

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(Plates I.-VIII.)

In a preliminary note communicated to this Society in September of last year I recorded the discovery of a species of Enteropneust at two widely separated localities on the coast of New South Wales, viz., at the ocean beach nearly opposite that part of Broken Bay known as Creel Bay, and also at Jervis Bay. Since the publication of the preliminary note I have found the same species on the beach nearly opposite Newport, N.S.W., and it may thus be looked for along the whole coast line of New South Wales. An examination of the form has shown that it is a new species belonging to the genus *Ptychodera*, and since it is the first

recorded from Australia, I propose for it the specific name australiansis.

The publication of Spengel's beautiful Monograph on the Enteropneusta* has rendered it unnecessary for me to enter into details of histology and the like, and also I do not propose, in this paper, to enter into the much discussed question of the affinities of the group. In the description, then, only points of specific difference are insisted on. I have adopted, generally, the terms proposed by Spengel, and my indebtedness to his monograph will be readily apparent from the sequel.

The species occurs in considerable abundance in a rocky corner of the ocean beach nearly opposite Creel Bay and a little to the south of the well known Hole in the Wall. Since the discovery of the species there in August of last year I have made two subsequent expeditions to the locality for further supplies of material, once in January and again in May of this year, and only on my last visit did I succeed in finding sexually mature individuals, so that the breeding season may, approximately, be given as the end of autumn.

During my visit to the locality I was very kindly accommodated by Mr. Chas. Hastie, of Creel Bay, and I must here take the opportunity of expressing my thanks to him for this kindness, and also to his family for much help in collecting specimens. Further, I must again acknowledge my indebtedness to Herr W. Musmann for much assistance with the literature.

Mode of Occurrence and External Characters.

Like the other species of the genus *Ptychodera*, *Pt. australiensis* is littoral and confined to very shallow water. It is found, at low water, most abundantly in the loose gravelly sand at the bottom of the shallow pools, and especially in such sheltered situations as the gravelly sand under and around the large stones occupying the area included between high tide and half tide marks, very few specimens (if any) being found close to the low water mark.

^{*} Fauna und Flora des Golfes von Neapel. xvii. Monographie: Enteropneusten, von Dr. J. W. Spengel.

The largest specimen found during my last collecting trip in May was a sexually mature male, measuring, in the living condition and when only very moderately extended, about 12 cm. in length with a breadth in the tail region of 7·25 mm. In August of last year, I found another large specimen which measured in the contracted condition about 18 cm. In the fully extended condition, this specimen, now in the teaching collection of the Biological Department of Sydney University, reached a length of over 25 cm. The majority of the animals were, however, very much shorter and thinner. They are capable of very considerable extension; for example, one specimen whose tail region had a transverse breadth of only 1·75 mm. reached in the fully extended condition a length of 11·7 cm.

Proboscis: The proboscis is relatively short like that of Pt. minuta and Pt. sarniensis. It varies in shape and length during life; when the animal is progressing it is more or less elongated, and when at rest generally somewhat egg-shaped, the latter being the shape it almost invariably takes when the animals are preserved in chrom-osmic acid. The proboscis of the first large specimen referred to above had a length of about 10 mm. in the living condition.

Collar: In the living animals the surface of the collar is smooth and in them, as well as in preserved specimens, it can readily be divided into the five characteristic regions (fig. 1). The first region includes slightly more than the anterior half of the collar, and is formed by the anterior free part of the latter. It spreads out anteriorly, investing the neck and base of the proboscis like a frill with margins crinkled as well during life as in preserved specimens. Behind the frill-like anterior region the collar is strongly contracted to form a well marked circular groove—the second region [figs. 1 and 15 (2)]—the anterior margin of which lies immediately above the mouth aperture. This groove is slightly more marked on the ventral side than on the dorsal.

The groove is followed by a prominent circular cushion of a lighter colour forming the third region [figs. 1 and 15 (3)]. Behind

this is found the typical ring furrow forming the fourth region [figs. 1 and 15 (4)], and this is separated from the trunk by a narrow projecting circular rim—the fifth region—which forms the posterior boundary of the collar, and is somewhat wider than the succeeding trunk section. The collar may reach a length of 9 mm. with a transverse breadth in the third region of 9 mm. also, so that in the living animal the collar may be said to be about as broad as long, but in preserved specimens the transverse breadth, owing to the strong contraction of the longitudinal collar musculature, considerably exceeds the length, and also the ventral length slightly exceeds the dorsal.

Trunk.

(1) Branchiogenital Region; This region may reach a length of from 36 to 40 mm. and a breadth of 7 mm. Pt. australiensis is especially characterised by the great development of the genital wings—the duplications of the dorso-lateral regions of the body in which the gonads are situated—and in this respect the species is to be associated with Pt. aperta, clavigera, gigas, and aurantiaca, in Spengel's provisional genus Tauroglossus. The anterior ends of the wings are continuous with the posterior rim of the collar, and immediately behind the latter their free edges may either slightly overlap each other or they may be infolded, and the wings then are in contact in the median line by their outer surfaces. The wings continue posteriorly in this condition and reach their maximum size somewhat posterior to the gill region. dition is retained, in a slightly varying degree in different individuals, to within a short distance of the most anterior liver sacs, which are more or less completely hidden from view by the wings. Beyond this point the wings decrease rapidly in size and form two ridges lying at first laterally to the liver sacs but which are finally covered by the outer overhanging ends of the larger liver sacs (fig. 24, gw.), and which disappear altogether in the posterior part of the hepatic region.

When the wings have the position just described no part of the gill area is visible externally, and the most anterior small liver sacs are also concealed from view. The gonads in sexually mature animals are found in the wings almost immediately behind the posterior rim of the collar, and they extend in the ridges far into the hepatic region. In sexually mature individuals the gonads may distend the genital wings to such a degree that they stand out almost horizontally to the body (fig. 23, gw.) and leave the gill area exposed, but the usual condition is the one first described where the gill area is completely concealed.

The gill area may reach a length of 15 mm., and is thus relatively very short. It is broadest just behind the collar, and narrows slightly posteriorly; laterally it is bounded by two longitudinal grooves which become gradually shallower posteriorly and terminate at the end of the gill area. Into these the gill pores open.

(2) Hepatic Region: varies in length according to the size and age of the animal. In one of my largest preserved specimens it measured over 20 mm, in length with about 50 liver sacs on each side. The number of sacs averages between 40 and 50 on each side, but there may be as many as 60. In fully extended animals the liver sacs present a distinctly paired arrangement in two longitudinal rows. They begin as small elevations in contact with each other, and covered by the genital wings; posteriorly they gradually increase in size, and attain their maximum development just beyond the point where the genital wings are reduced to ridges. Behind this the sacs gradually decrease in size to the posterior end of the region, finally being represented by two rows of small tubercles which become gradually reduced until they disappear altogether. Each sac arises by a narrow base which widens out into an anteroposteriorly compressed end, which in the region of the larger sacs overhangs the reduced genital wings. The form of the sac in this region is shown in fig. 24, hep.

Variation in Hepatic Region. The sacs are usually in close contact with each other by their anterior and posterior faces, but occasionally some of the caeca present the

appearance as if they were pressed out of position and overhang the sides of the body more than the others, giving the rows a slightly irregular appearance. I have also met with two specimens in which as a further result of displacement two rows of alternating sacs were formed on each side in the middle part of the hepatic region. Both these specimens had lost the whole of the body anterior to the liver sacs, and a new proboscis was in course of formation at the anterior end of the hepatic region. In a third specimen the sacs were normal in arrangement up to the larger sacs, but the succeeding sacs presented a very irregular appearance, forming two lateral masses, composed of irregularly arranged sacs, on either side of the dorsal median line, and not showing the alternation of the sacs seen in the other two specimens. It might be that this irregularity is the result of regeneration of the posterior part of the body.

(3) Tail Region: varies greatly in length in different individuals, but is usually about three times as long as the hepatic region. Its outline varies according as it is empty or full of sand; in the former case it is flattened, and in the latter more or less tubular.

The median dorsal line in preserved specimens is occupied by a dark ridge extending to the posterior end of the body, and marking the position of the dorsal nerve stem; on the ventral side the ventral nerve stem occupies the bottom of a shallow longitudinal groove. The tail region in this species is especially characterised by the presence of two dorsally situated longitudinal epidermal stripes (fig. 2) running parallel with the dorsal nerve stem and at a short distance from it. Appearing in the hepatic region just external to the posterior small liver sacs they extend over about the anterior two-thirds of the tail region, and are situated immediately above the ciliated grooves of the intestine. The epidermal stripes can be distinctly seen not only in preserved specimens but also in the living animal. Indeed, I observed and figured them in the latter before I knew of the existence of the two ciliated grooves in the intestine. The two epidermal stripes appear as two longitudinal interruptions of the island-like groups of epidermal gland cells. The groups occupying the dorsal surface of the body extend between the upper margin of the epidermal stripe and the dorsal median line, while those occupying the ventro-lateral regions of the body terminate on its lower margin. According to Spengel, a similar epidermal stripe exists in Pt. minuta, but in correspondence with the single ciliated groove on the left side, only on that side, so that the presence of two epidermal stripes in Pt. australiensis may be taken as a character of specific value.

Behind the termination of the epidermal stripes the tail region becomes gradually somewhat narrower to its posterior end, in which is situated the terminal anus of varying outline.

Colouration: The sexually mature males and females can very easily be distinguished from each other by their different colouration. In both sexes the proboscis is of a light yellow colour, while the collar is of a slightly deeper yellow. In the males the testes are of a very deep yellow colour, or less frequently in some individuals of a deep orange. In the females the ovaries are of a very light yellow or almost whitish colour. The females are on the whole of a lighter colour than the males.

The most anterior liver sacs are of a brownish colour which passes posteriorly into slaty green, and this again into a deep brown in the region of the larger caca, this brown colour being retained more or less completely in preserved specimens, while the other sacs lose their colour entirely. Posteriorly the sacs again assume a slaty green tinge. The tail region is whitish in colour and its walls transparent, readily allowing the sand particles in the intestine to show through.

This species is destitute of any odour. Incidentally it may be here mentioned that a large proportion of the individuals of this species are infested by a parasitic Copepod belonging to the genus *Ives* of Mayer,* but whether it is identical with the single member of the genus hitherto described, *Ives balanoglossi*, I am

^{*} Ein neuer parasitischer Copepode. Mitth. Zool. Stn. Neap. Bd. i. p. 515.

as yet in doubt. The large female with its attendant small males is usually found in a very prominent tubular enlargment of a part of the free edge of one of the genital wings.

ANATOMY.

Proboscis: The epidermis of the proboscis (fig. 5, ep.) is somewhat thicker than in the case of Pt. minuta measuring 11 mm. It is separated from the underlying musculature by the limiting membrane, composed of two layers, the outer attached to the nerve fibre layer of the epidermis, the inner to the circular muscular layer. Between these two layers run the capillaries of the vascular net of the proboscis (fig. 5, cnp.). Below the limiting membrane is the thin layer of circular muscle fibres, also somewhat thicker than in the case of Pt. minuta, viz., 023 mm. circular muscular layer gradually becomes thinner towards the neck of the proboscis, and finally thins out altogether. delicate membrane limiting the circular muscular layer internally can readily be recognised especially in horizontal sections. On the inner side of the membrane, and at a little distance from it, there is an irregular cell layer, the nuclei of which are very distinct in hæmatoxylin preparations.

From the outer side of this limiting membrane there pass through the circular muscular layer numerous fine fibres to the inner layer of the limiting membrane of the proboscis, while on its inner side the fibres of the longitudinal musculature are inserted.

The fibres of the longitudinal musculature are related in their mode of origin from the proboscis base and in their course from there exactly as in *Pt. minuta*, and they show the same separation in preserved specimens into radial masses (fig. 5, *lm.*). The muscle fibres are embedded in a very fine connective tissue which anterior to the proboscis organs occupies the central region of the proboscis, muscle fibres being there absent. The connective tissue here presents a spongy appearance with a few very small nuclei situated in the course of the connecting strands or in the angles between the meshes. The splanchnic epithelium of the proboscis

cœlom (figs 4, 6, 7, 8, sp.) is related exactly as in the described species. It appears, in my preparations, when it comes to lie directly on the sides of the proboscis gut as a deeply staining layer with dark staining nuclei in its middle region (fig. 7, sp.), the cell bodies of which are not recognisable. On the surface of the glomerulus it presents essentially the same appearance of a deeply staining granular layer with numerous dark staining nuclei on its inner face (fig. 6, sp.).

On the outer side of the splanchnic epithelium there is a layer of tissue of a spongy appearance (figs. 6 and 7, ct.) which is directly continuous both above and below with the fine connective tissue of the proboscis ceelom (fig. 4, ct.). This layer corresponds to the characteristic layer described by Spengel* on the surface of the glomerulus in Pt. sarniensis, and which he considers as the inner limiting layer of the connective tissue of the proboscis. Round the central proboscis organs there is always in sections, as in the described species, a space free from connective tissue and representing the proboscis colom (figs. 5 and 8, pbc.). towards the proboscis neck the proboscis colom is divided, as in the other species, on the dorsal side through the heart bladder into two dorsal proboscis pockets (fig. 9, dp.), and on the ventral side through the ventral septum into two smaller ventral proboscis pockets (fig. 9, vp.). The ventral septum (figs. 3-5, 8, vps.) has in this species anterior and posterior free edges which run obliquely backwards, while its dorsal and ventral edges are attached to the proboscis gut and the outer wall of the proboscis respectively so that the septum is here four-sided and not triangular as in Pt. minuta. In its structure it is essentially similar to that of Pt. minuta. It consists of a central membrane carrying blood vessels, on either side of which there is a layer of muscle fibres—the ventral portion of the fibres of the dorso-ventral muscle plate; on the dorsal side, the fibres of the same plate can be distinctly recognised on the lateral walls of the heart bladder, below the splanchnic epithelium (figs. 3 and 5, dsc.).

The splanchnic epithelium continues on the surface of the ventral septum which, as Spengel has shown, represents a median fold of the ventral proboscis wall, and is connected at intervals with the limiting membrane of the epidermis. The two ventral proboscis pockets separated from each other by the ventral septum are usually filled by a loose connective tissue; however, in some cases, a distinct space bounded by this tissue may appear (fig. 12, pp.) Behind the posterior free edge of the septum the ventral pockets unite to form the ventral unpaired portion of the proboscis celom.

Exceptionally two folds may arise from the limiting membrane of the epidermis to form the proboscis septum; the two folds enclosing a median pocket between them unite with each other along the mid-ventral line of the proboscis gut. Further posteriorly the point of union of the two folds gradually passes ventrally until the median pocket is obliterated, and the septum assumes the normal condition. On the dorsal side the two proboscis pockets separated by the heart bladder are in different individuals of this species very varied in their relations. The proboscis pore may be single or double, and occupy a median position or it may be single and situated on the right side of the median line.

- (a) In the majority of individuals the proboscis pore occupies a median position (fig. 12, p.) and it may then be formed in three ways—(1) The two dorsal proboscis pockets may unite with each other behind the heart bladder to form a single median proboscis canal which opens by the median proboscis pore; (2) the left pocket alone may form a proboscis canal, the right ending blindly; (3) the right pocket may form the canal, the left ending blindly.
- (b) There may be two proboscis pores, the two dorsal pockets giving rise to two proboscis canals (fig. 10, pc.) which both open to the exterior (fig. 11, p.).
- (c) The proboscis pore may be single and open on the right side; in this case the left pocket ends blindly while the right continues posteriorly, taking an almost median position above the

heart bladder to form the proboscis canal which opens on the right side.

With regard to the behaviour of the dorsal proboscis pockets, *Pt. australiensis* appears to be the most variable of all the Enteropneusts hitherto described.

Notochord (Eichel-darm of Spengel): In shape the notochord of this species essentially resembles that of Pt. minuta. Immediately anterior to the point of opening of the lumen of the notochord into the mouth or throat cavity, its narrow neck portion is dorso-ventrally compressed, with a convex dorsal wall and a concave thin ventral wall composed of a single layer of low columnar cells resting on the proboscis skeleton (fig. 16, div.). Anteriorly in the region of the proboscis neck the neck portion of the notochord is not so much dorso-ventrally compressed, but somewhat higher and with a more or less triangular lumen. The dorsal wall of the neck portion of the notochord is very much thicker than the ventral and is composed of long narrow epithelial cells which radiate outwards from the lumen and have central generally narrow rod-like nuclei. Between these elongated cells there occur numerous clear oval bladders which Spengel well regards as the secretory holders of gland cells. Some appear quite empty, others again show a network in their interior similar to that in the epidermal mucous glands and which stains in the same diffuse manner. They thus conform, as Spengel has shown, to the structure of the "goblet cells;" on the ventral side where the wall is composed of a single layer of columnar cells, these gland cells are entirely absent. The neck portion of the notochord is thus distinctly epithelial in character.

Anteriorly the dorsal wall increases considerably in thickness, while the cells of the ventral wall lose their distinctly columnar quality. They become longer and narrower, gland cells appear between them, and they finally pass over into the chorda-like tissue forming the wall of the ventral blind sac of the notochord. The lumen of the notochord extends obliquely downwards into the ventral blind sac (fig. 14), then in this the lumen extends,

transversely outwards, reducing the lateral walls of the blind sac to a thin layer, while the hind and middle portions of the wall are somewhat thicker, as in Pt. minuta. From the transverse lumen of the blind sac there passes forwards laterally a short horn on each side (fig. 9, lb.); a section passing through the proboscis neck just anterior to the passing down of the ventral blind sac lumen thus shows three cavities in the notochord (fig. 9), two ventrolateral belonging to the horns of the ventral blind sac lumen and a median situated near to the dorsal side of the notochord, the main notochordal lumen now considerably reduced in size and with numerous gland cells opening into it. Anteriorly the lumen comes to occupy a more nearly central position (fig. 8, div.); it finally becomes reduced to a narrow slit (fig. 4, div.), which can be traced to a slightly varying distance from the apex of the notochord (fig. 14, div.). The lumen varies somewhat in position and shape in different individuals, being in some situated nearer the dorsal side, in others nearer the ventral side of the notochord; its outline also varies—it may be circular or form a narrow vertical or horizontal cleft. The lumen in this species has on the whole a more median position than in Pt. minuta, and consequently the dorsal and ventral walls are more nearly equal in thickness. In fig. 14 the lumen of the notochord is represented somewhat diagrammatically as a continuous wide canal, but as in Pt. minuta it is interrupted by occasional bridges of tissue, and also as in that species the dorsal wall of the lumen is smooth, while into the ventral there project short processes of the lumen (fig. 14, div.). With the exception of the anterior part of the lumen numerous gland cells open into it, testifying to the epithelial nature of the whole structure. Numerous gland cells exist in the dorsal wall of the neck portion of the notochord and they extend for a considerable distance into the head region, being specially abundant round the part of the lumen situated just anterior to the point of origin of the ventral blind sac lumen. The lumen is, in some preparations, almost filled up by a diffusely staining network, apparently derived from the secretion of the gland cells opening into it. Numerous elongated cells, between which the gland cells are situated, radiate outwards from the lumen. They have elongated, rod-like nuclei situated at about their middle region, and on the dorsal side their outer ends come into connection with processes from an irregular layer with rounded nuclei situated immediately below the limiting membrane of the notochord. On the ventral side the passing over of these distinct epithelial cells into the chorda-like tissue can be distinctly recognised (figs. 9 and 12, dv.).

In correspondence with the widely diverging legs of the proboscis skeleton the opening of the proboscis gut lumen into the mouth cavity, at first narrow, becomes eventually a very wide transverse opening, in the anterior part of the mouth cavity. I have also encountered in the proboscis gut the yellow granules which Spengel regards as excretions.

Proboscis Skeleton: it consists, as in the described species, of a main body, a keel-like ventral portion, and two posterior diverging legs (fig. 13). The anterior portion of the body is formed by the funnel-like "end plate" which invests the ventro-lateral regions of the ventral blind sac of the notochord. The lateral edges of this plate extend somewhat in front of the ventral, so that they appear first in sections as two thin plates investing the lateral faces of the blind sac (fig. 9, eps.). anterior "end plate" gradually narrows posteriorly, its dorsal edges unite with each other in the median line, and it passes into the body proper. By the union of the dorsal edges of the "end plate" there is sometimes formed a blind canal continuing for a short distance into the body proper, and occupied by a prolongation of the "chorda-like tissue" of the hind wall of the ventral blind sac. The "keel" first appears at a short distance behind the anterior edge of the "end plate" in the form of a small V-shaped structure with widely divaricated legs. It is separated from the end plate by a thin band of "chondroid tissue." Then gradually the ventral apex of the keel thickens and gives rise to a distinct ventrally projecting tooth-like portion, the keel now having in section the form of a Y (fig. 10, kps.). The "body" has at first a semilunar outline, with the flat side immediately adjoining the ventral wall of the neck of the notochord and the curved side above the divaricated legs of the Y-shaped "keel." At this point there passes in the "chondroid tissue" between the "body" and "keel" a vessel connecting the two efferent proboscis vessels (fig. 10, cv.) Immediately behind this vessel the "body" becomes triangular in outline and its apex gradually approaches and finally fuses with the median portion of the "keel" between its divaricated legs which now give rise to two lateral "wings." Anterior to and at the point of fusion the body is much stouter than the keel, the "wings" of the latter only projecting a very little beyond the lateral surfaces of the body, but posterior to this the body gradually decreases in breadth and also in height, while the keel thickens greatly, forming in transverse sections much the bigger half of the whole structure. The "wings" of the "keel" at the same time reach a greater lateral extension and form two distinct lateral projections below the middle region of the body when the entire skeleton is viewed from above (fig. 13). Posteriorly the wings become gradually smaller and finally disappear, while the body becomes reduced to a narrow somewhat convex plate separated from the keel by two small vertical half moonshaped masses with their convex faces touching each other. These are the first indications of the two "legs," and for them Spengel adopts Bateson's term "nuclei." After the first appearance of the "nuclei" the keel gradually becomes reduced in size, the "nuclei" at the same time becoming larger and more distinct. The place where the "nuclei" first touch in the middle line corresponds, as Spengel has shown, to the most anterior point the opening of the notochordal lumen into the mouth cavity has occupied. In transverse section the proboscis skeleton has, just after the proboscis neck has fused with the collar, a triangular shape, but gradually as the "keel" is reduced in size and the "nuclei" become larger and more distinct, the shape becomes quadrangular and the skeleton then consists of a dorsal plate, representing a continuation of the "body" and derived from the notochord, a middle portion formed by the two semilunar "nuclei" derived from the throat epithelium, and a ventral plate thicker in the middle—the continuation of the "keel"—derived also from the throat epithelium as the opening of the notochordal lumen gradually moved posteriorly (fig. 16, lps.). Towards the posterior end of the skeleton the "keel" disappears entirely, the two "nuclei" separate from each other ventrally, and the epithelium of the throat extends up into the cleft between them. The continuation of the body lying above the nuclei then thins out, and the "nuclei" separate completely from each other, giving rise to the "legs" proper which lie under a fold of the throat epithelium. The "legs" may pass out at once almost transversely, or they may diverge more gradually in different individuals. They terminate considerably in front of the middle region of the collar.

The proboscis skeleton is composed of a cuticular substance which shows in this species a very distinct stratification, indicating the mode of origin of the mass by the deposition of successive layers, and the direction of these layers indicates very clearly from what source they are derived. As Spengel has shown, the "end plate" is derived from the ventral blind sac of the notochord, while the "body" is derived from the neck of the same. The "keel" on the other hand is formed from the epidermis of the proboscis neck, and its posterior continuation from the epithelium of the throat.

In this species the "end plate" and the anterior portion of the "body" are separated from the keel by chondroid tissue, and the direction of the lines of stratification in these parts indicates clearly enough their distinct origin. The relations of the skeleton to the limiting membranes are essentially those of the described species. The body passes over at its edges into the limiting membrane of the notochord, while the wings of the keel pass over into that of the epidermis.

In the proboscis skeleton of this species there occur a few small oval cells with non-staining cell bodies and deeply staining small nuclei. They are situated between the layers of stratification. Such cells occur in the proboscis skeleton of *Pt. clavigera*, *gigas* and *aurantiaca*, and with Spengel I believe they are cells

which have become enclosed during the formation of the skeleton, and not cells which have immigrated later.

"Chondroid tissue": As in the genus Ptychodera generally the "chondroid tissue" of the proboscis neck is not greatly developed, and the cell strands appearing generally in transverse sections as small isolated masses are derived as Spengel has shown mainly from the epithelium of the proboscis pockets. As in Pt. clavigera a band of "chondroid tissue" continuous with the lateral tissue is present between the "end plate" and "keel" of the proboscis skeleton. The cell strands of this ventral portion are very richly developed, and are derived from the epithelial cells lining the ventral proboscis pockets and behind the posterior edge of the proboscis septum from the continuation of the same epithelial cells lining the ventral unpaired portion of the proboscis cœlom.

Heart-bladder: The heart-bladder is esentially similar in its general relations to that of Pt. minuta. It is a completely closed sac, having no connection either with the vascular system or with the proboscis colom. On its lateral walls the muscle fibres belonging to the dorso-ventral muscle plate are very well marked (fig. 5, dsc.), but as in the other species of the genus they do not possess a musculature of their own. On the ventral wall there is present as in the described species a very distinct single layer of transverse muscle fibres which, so far as I have observed, are entirely confined to this wall. In this species the ventral wall of the heart-bladder is infolded into the cavity of the bladder in a very characteristic manner. In its posterior part the central blood space of the proboscis is a transverse cleft between the ventral wall of the heart-bladder and the proboscis gut just as in the species previously described, and varies in size according as it is filled with blood or empty. In its anterior region, however, the ventral wall of the heart-bladder is infolded into the cavity of the bladder along the median line so as to give rise to a tubular cavity which communicates with the central blood space by a narrow longitudinal slit (fig. 4, ivw.). Then posteriorly to the infolding by the gradual receding of the two edges of the slit, the tubular cavity merges gradually into that of the central blood space. Anteriorly, however, the anterior end of the tubular infolding projects towards the end of the heart-bladder as a short free blind sac which extends into the cavity of the heart-bladder beyond the anterior end of the longitudinal slit placing the tubular cavity in communication with the proper cavity of the central blood space. Consequently in a series of transverse section this free end first appears as an apparently isolated cavity with muscular walls lying in the cavity of the heart-bladder and quite independent of its ventral wall (fig. 3, ivw.).

The only parallel for this condition in other Enteropneusts appears to exist in *Balanoglossus canadensis*, which, according to Spengel, possesses a similar infolding of the heart-bladder wall, although it is still more complicated in other respects.

According to Spengel the ventral wall of the heart-bladder serves to furnish the central blood space with the musculature by whose contraction the blood is forced out of the central blood space into the sinuses of the glomerulus, and thence into the efferent proboscis vessels. We have therefore to regard this infolding of the ventral heart-bladder wall into a tube free anteriorly, inasmuch as it increases the power of that wall, as a special modification to ensure the better performance of its propelling function. I have found this condition so frequently in this species that it may be taken as a character of specific value.

In the interior of the heart-bladder a space exists in its greater extent (figs. 3, 4, 5 and 14, h), but at its anterior and posterior ends the cavity is obliterated by a cellular tissue. Anteriorly this tissue has the appearance of a spongy connective tissue with numerous nuclei in its connecting strands, and I have not been able to observe in this any distinction into two portions, one derived from the dorsal, the other from the ventral wall, such as Spengel describes for $Pt.\ minuta$. An irregular cavity appears in the dorsal part of this mass, a short distance behind the anterior end of the heart-bladder, and the loose tissue below it gradually becomes reduced in size passing into an irregular layer of endothelial cells on the ventral wall of the heart-bladder. These

cells do not form in this species, so far as I have observed, a definite layer, but are irregularly disposed (figs. 4 and 5), and very frequently some of them are of an elongated form with one end attached to the heart-bladder wall. The portion of the anterior cell mass above the cavity continues as a narrow strand occupying the apex of the cavity of the heart-bladder, and limited from it by a very tender membrane (fig. 5). This dorsal strand of tissue passes on either side into the flattened endothelium which lines the lateral walls of the heart-bladder. The rounded nuclei of this endothelium can be readily recognised, placed at fairly regular intervals from each other, but the cell bodies are not distinct in my preparations. As the heart-bladder decreases in size posteriorly the cells of the dorsal strand unite with processes from the irregular endothelial cells of the ventral wall, and eventually the posterior part of the cavity is filled up as in Pt. minuta by a mass of tissue denser than that of the anterior end (fig. 9, h.), and through which there pass between the lateral walls of the heart-bladder fine transverse fibres which Spengel regards as muscular.

Yellow granules may sometimes be observed in the cellular tissue in the heart-bladder.

Glomerulus: The glomerulus will be dealt with in connection with the vascular system, and I need only mention here one point in connection with it. Lying on the splanchnic epithelium and internal to it between the glomerulus vessels I have observed, in some individuals of this species though not in all the series I have examined, some bundles of parallel muscular fibres (fig. 6, qlm.). They very often have the appearance of being inserted into the tender membrane forming the walls of the vessels of the net at the periphery of the glomerulus. The fibres also frequently project beyond the outer surface of the splanchnic epithelium, and readily give one the impression that they were during life continuous with similar bundles which lie at the inner edges of the radial masses bounding the space surrounding the proboscis organs, and from which they are separated only by a very narrow These muscular fibres in the glomerulus of this species interval.

appear to correspond to the fibres Spengel has observed in *Balanoglossus canadensis* and *B. kupfferi*, and which he regards as having been carried in, by the infolding of the splanchnic epithelium.

Collar: The epidermis of the collar can, like that of the other species of Ptychodera, be divided into five zones (fig. 15 [1-5]). Of these the first formed by the anterior free rim of the collar is the largest; the second occupies the region of the circular groove; the third, that of the circular projecting cushion; the fourth, the bottom of the typical ring furrow; while the fifth is formed by the narrow projecting rim forming the posterior boundary of the collar. The second and fourth zones are similarly constituted; they stain darkly with hematoxylin, and as in Pt. minuta they contain in their whole depth numerous gland cells; the remaining zones contain gland cells only in their outer regions, and stain of a lighter colour.

Collar musculature: It is in this species in its general relations essentially similar to that of Pt. minuta.

In the outer wall of the anterior part of the collar there is the usual external layer of longitudinal muscle fibres (figs. 14, 16, lfw.) which spring in the posterior part of the collar between the longitudinal muscle bundles of the inner wall. Internal to these there is the layer of circular fibres which terminate at the beginning of the second epidermal zone (figs. 14, 16, cfiv.). The longitudinal muscles of the inner wall of the collar springing from the region of the collar trunk-septum are, as in Pt. minuta, separated in the posterior part of the collar into distinct bundles by radial fibres passing between the outer and inner walls of the collar. The ventro-lateral bundles terminate behind the circular vessel fold of the collar, while those more dorsally situated pass towards the neck of the proboscis, and are inserted, as in Pt. minuta, mainly into the boundary membrane of the notochord (fig. 16, ilm'.; fig. 11, ccp.). The dorsal portion of the longitudinal musculature extends, in the posterior region of the collar, round the lateral surfaces of the perihamal spaces, and partly on to the dorsal surface of the collar cord. Anterior to the circular vessel fold there occurs a fairly thick band of muscle fibres which arise from the sides of the proboscis skeleton, and surround the mouth aperture circularly, and from this layer there pass up the longitudinal fibres of the fore wall of the collar (fig. 14, ifw.). The radial fibres passing between the fore and outer walls of the anterior margin of the collar have the usual intercrossing arrangement (fig. 14, rf.).

The extensions of the trunk colom into the collar—the perihæmal and peripharyngeal spaces—are related essentially as in the known species of Ptychodera. As in Pt. sarniensis and aperta, the perihemal spaces, with the exception of their anterior portions which are situated entirely below the collar cord (fig. 16, phs.), enclose between them a groove in which the ventral two-thirds of the collar cord is situated (figs. 17 and 18, phs.). The greater portion of their cavities is occupied by the longitudinal musculature of the dorsal wall, which is inserted anteriorly in greater part into the boundary membrane of the epidermis behind the proboscis canal. On the ventral wall of each space there is a single layer of longitudinal fibres, while between dorsal and ventral walls there pass radial fibres. The peripharyngeal space (figs. 17, 18, pps.) is related exactly as in Pt. minuta. As in that species, there pass across the circular fibres which it contains numerous connecting strands between its inner and outer limiting Anteriorly it terminates on the dorsal side about on membranes. a level with the opening of the notochordal lumen into the throat (fig. 14, pps.), while ventro-laterally it terminates along the point of origin of the circular vessel fold.

Collar Cælom: In the anterior part of the collar the spongy connective tissue containing radial muscle fibres fills up the celom almost completely, but posteriorly where the fibres are arranged in the form of radial strands there remain between adjacent strands spaces free from connective tissue just as in Pt. minuta (fig. 18, cæ). Ventrally a longitudinal space exists, into which the ventral vessel fold projects, and on the dorsal side, just

anterior to the internal openings of the collar canals, two distinct spaces exist.

With regard to the division of the collar colom into two halves, considerable variation exists in this species. Dorsal and ventral septa may be entirely absent, the two side halves of the colom then standing, as in *Balanoglossus kupfferi* according to Spengel, in open communication.

The dorsal septum when present differs from the normal condition in Pt. minuta with regard to its relation to the "roots." In that species, according to Spengel, the dorsal septum only exists in the posterior part of the collar; two "roots" are situated anterior to it, and its anterior free edge always appears to correspond to one of the "roots." In this species the septum may arise in the anterior region of the collar and in front of the most anterior root as a fold projecting from the dorsal surface of the collar cord and with a free anterior edge, and which reaches the outer wall where the first "root" fuses with the collar epidermis. The septum then passes between the remaining "roots" to the posterior end of the collar. Over part of its course it may be interrupted: the ventral connection with the dorsal surface of the cord is lost, the septum at the point of interruption appearing as a free fold projecting from the inner surface of the outer collar wall. Further, in other individuals the front edge of the septum may coincide with either the first or the second "root."

As to the ventral septum (fig. 19, vcs.) when present, it exists only in the most posterior part of the collar and there only for a very short distance. It may be present when the dorsal is absent. In fig. 18 a section passing just anterior to the ventral septum is represented. The vessel fold (s/v.) arising from the subepidermic collar capillaries exists only for a short distance in the posterior region of the collar, and is not always so distinct as in the series from which this section is taken; posteriorly it unites with one of the longitudinal vessel folds to give rise to the ventral septum (fig. 19, vcs.). The collar canals and the collar-trunk septum are related in this species exactly as in Pt. minuta.

Nerve Cord of Collar: The collar nerve cord (figs. 16, 17, 18, cnc.) varies in shape in transverse sections in different individuals and in different parts of the same individual, from band-shaped to almost circular. In its general features the cord of this species agrees with that of Pt. minuta as described by Spengel. An axial canal is not present in the cellular part of the cord, and the "cord hollows" are not so numerous as in that species. Two lateral longitudinal rows of "cord hollows" exist, while smaller, less regularly arranged hollows may be present in the central region. The entire number of hollows appearing in one section is seldom greater than four. These "cord hollows" are related to each other exactly as in Pt. minuta.

I have never observed an anterior insinking of the epidermis into the cellular part of the cord to form an anterior epidermal pocket, but a very short and narrow epidermal pocket may exist at the posterior end of the cord.

As in all other known species of the genus, the fibrous layer of the cord completely surrounds the cellular portion.

With regard to the histology of the cellular part of the cord, I have never been able to observe in any of my preparations the giant ganglion cells described and figured by Spengel. I can clearly distinguish, however, large granular nuclei, rounded or oval in form, and with distinct nucleoli, which belong probably to what Spengel regards as the proper nerve cells. They occur especially above the ventral portion of the nerve fibre layer and also in the middle region of the cord and below the thin dorsal portion of the fibre layer. Round these nuclei there may sometimes be seen an ill-defined little staining cell body, apparently branching.

The "stalked cells," with their elongated, deeply staining, narrow nuclei can also be readily distinguished. They radiate outwards from the thin cuticular lining of the "cord hollows," and their fibre-like ends penetrate the nerve fibre layer. Also there occur throughout the cellular part of the cord, but especially above the ventral portion of the nerve fibre layer, numerous deeply staining rounded nuclei, with ill-defined cell bodies.

In the nerve fibre layer, below the cellular part of the cord, nuclei are found, with small oval non-staining cell bodies which are produced at either end into fibre-like processes. Clear oval spaces also occur in the fibrous layer, but I have not been able to convince myself that these form continuous tubes, and they never possess any protoplasmic remains in their interior such as Spengel describes for the processes passing into the fibrous layer from the giant ganglion cells. These spaces appear to be simply lacunæ between the ramifying fibres composing the fibrous layer.

Roots: In this species the most anterior "root" may arise from the anterior region, i.e., in front of the middle point of the collar cord, contrary to Spengel's statement that the roots always spring from its middle or posterior end. Generally, however, the first root is situated about the middle region of the cord. The roots vary in number from one to four, the latter number being the usual one. In one series five roots arose from the collar cord, but I was unable to find the dorsal point of union of the fifth with the epidermis. As in the described species, the roots vary greatly in direction; sometimes they pass straight between the collar cord and the epidermis, but generally they take an oblique course, forwards or backwards, to their point of union with the epidermis. In one series the first two roots fused with each other midway between the collar cord and epidermis, while in another series two of the roots arose from the collar cord by a short common stem. Each root consists as in the described species of an outer limiting membrane carrying blood vessels, and continuous at the one end with the limiting membrane of the dorsal cord, at the other with the limiting membrane of the epidermis. Internal to this is a thin fibre layer continuous ventrally with the nerve fibre layer of the collar cord. The interior of the root presents a varying appearance at different parts; in some sections, and especially in those of the first root, a distinct central cavity may exist limited by a delicate membrane from which processes radiate outwards. These processes apparently belong to cells, the nuclei of which are situated just internal to the fibrous layer, the whole structure recalling the appearance of the stalked cells radiating

from the "cord hollows." I have never been able to trace these hollows in the roots as continuous canals throughout the whole length of even the first "root," and am convinced that in none of the "roots" of this species does a continuous canal exist such as Spengel describes for the first "roots" in Pt. minuta, clavigera, aperta, and bahamensis. Similar though smaller hollows may exist in the "roots" succeeding the first, but just as in the first "root" the hollows do not stand in continuous connection, but are interrupted by anastomosing strands or in some cases by branching cells with large nuclei. I have likewise never observed the "collar hollows" to pass into the roots.

The roots end dorsally just as Spengel describes (fig. 16, rt.). The outer limiting membrane and the fibrous layer of the root unite with the similar layers of the epidermis; while the cellular tissue of the root projects in a cone-like mass between the epidermal cells, the apex of the cone reaching to within a short distance from the outer surface. The apex of this cone-like mass of tissue is composed of a network of fine strands enclosing clear spaces, and with a few very small nuclei in the angles where the strands meet. Below this clear tissue and continuous with it, there occur more deeply staining branched cells which are directly continuous with the central tissue of the "roots."

Nerve Stems of Trunk: These are related exactly as in the described species, and the circular commissure placing the fibrous layer of the collar cord in connection with that of the ventral stem can be readily seen in horizontal sections occupying the deep groove between collar and trunk. The cells covering the circular fibre ring are entirely destitute of gland cells, and consequently are sharply marked off from the adjacent epidermal cells.

The dorsal stem in the branchial region is situated somewhat below the level of the adjacent epidermis (fig. 20, dnv.), but posterior to this region it forms a distinct ridge. The ventral stem, on the other hand, occupies over its whole extent the bottom of a shallow longitudinal groove (figs. 20, 24, vn.). The fibrous layer of the dorsal stem is, as in Pt. minuta, somewhat triangular in transverse section; it is thickest in the middle, and laterally

tapers off gradually into the fibrous layer of the epidermis. That of the ventral stem is more band-like in form, and laterally passes over suddenly into the epidermic fibrous layer. In the cellular portion of the dorsal stem gland cells are comparatively few in number, while they are altogether wanting in the ventral. Of the two stems the ventral is the more strongly developed, and towards the posterior end of the body it remains more distinct than the dorsal.

In my preparations, and especially in the ventral cord, the "stalked cells" of Spengel can readily be distinguished. are elongated fibre-like cells with generally long deeply staining nuclei. These cells are specially developed at the lateral margins Their fibre-like basal processes traverse the of the ventral stem. fibrous layer, while their outer ends converge towards the middle line of the stem, thus enclosing a central space largely occupied by the nerve cells. The nuclei of these latter are easily distinguishable by their large size and rounded appearance; they do not stain very deeply, possess distinct nucleoli, and exactly resemble the nuclei of the nerve cells in the collar cord. The nerve cells lie immediately adjacent to the fibrous layer, and this fibrous layer as well of the collar cord as of the trunk nerve stems is to be regarded, according to Spengel, as made up of the ramified and interlaced processes of these nerve cells. Here, as in the collar cord, I have never succeeded in observing the giant ganglion cells.

Trunk.

(1) Branchial region: As in all known species of Ptychodera there is below the epidermis a delicate layer of circular muscle fibres, in this species only a single fibre in thickness. The longitudinal musculature internal to this is strongly developed and as in the described species is interrupted dorsally and ventrally by the vessel stems. The longitudinal musculature of the outer wall of the genital wings is considerably stronger than that of the inner wall. The latter is interrupted in the region of the sub-median lines in whose course the openings of the ducts of the gonads are situated. Numerous radial fibres

pass from the limiting membrane of the epidermis through the muscular layers to be inserted into the ventro-lateral portions of the walls of the esophagus and into the lateral regions of the gill pockets. Laterad of the esophagus a free space is left as in the described species, and this contains in sections a coagulated fluid in which cells are embedded. The two halves of the trunk celom are completely separated from each other ventrally by the ventral vessel which extends between the limiting membrane of the epidermis and that of the gut. On the dorsal side the dorsal vessel may occupy the whole height of the mesentery or only its dorsal half.

Gut Canal of Branchial region: As in all the known species of the genus Ptychodera the gut canal in this region is separated into two passages—a dorsal or branchial canal (fig. 20, gg.) and a ventral or esophagus (fig. 20, e.). They communicate with each other by a narrower or wider cleft according as the two limiting cushions (fig. 20, lc.) are approximated to or remote from each other. Except for the much greater development of the genital wings in this species, a transverse section through its branchial region (fig. 20) agrees almost exactly with a similar section of $Pt.\ minuta$. The gill skeleton is composed of three-pronged forks which are related just as in $Pt.\ minuta$; the number of "Synaptikels" (fig. 20, e.) varies from seven to ten.

The epibranchial stripe forming the median dorsal boundary of the branchial canal is composed of numerous relatively very long and narrow cells; and its middle region does not stain so deeply as the lateral regions in which gland cells are situated. The lateral regions pass over opposite the "gill tongues" into the high epithelium (fig. 21, gtb.) of the "tongue back" (the inner wall of the tongue next the gut canal). The gland cells are specially abundant in the middle region of the epithelium of the "tongue back," and not placed nearer its front face as in Pt. minuta. This epithelium passes over into the narrow strand of "intermediate epithelium" (fig. 21, ies.) composed of small non-ciliated cells which furnishes the low epithelial covering of the "Synaptikels." The lateral walls of the tongue and of the gill pocket are lined

by a layer of columnar cells with long cilia just as in the described species, and the "tongue floor" composed of cells poor in protoplasm is also, as in them, infolded into the cavity of the "tongue." This cavity (fig. 21, gtc.) just as in Pt. minuta is lined by an irregular peritoneal layer, and also contains fibres which stretch across between its opposite walls at a short distance below the "tongue back."

The gill pores (fig. 20, gp.) are narrow slits slightly obliquely placed, which open into the longitudinal grooves bounding the "gill area" laterally, and as in the described species the longitudinal muscles lying laterally to the pores give off fine bundles which pass between adjacent pores to join the longitudinal muscles on the inner side of the gill pores.

The anterior ends of the gill rows project for some distance into the posterior region of the throat (fig. 18, ag.), but owing to the oblique direction of the most anterior gills the posterior rim of the collar forming the so-called "operculum" of Bateson covers at most only the first gill pore. The conditions are essentially the same as in Pt. clavigera since the genital wings do not possess free anterior edges, but are united with the hind edges of the collar, thus enclosing between them and the "gill area" the space for which Spengel adopts the term "atrium." It is into the narrow anterior projection of this space below the posterior edge of the collar that the first gill pore opens.

The number of gills varies according to the age of the animal, since new ones are being continually formed during life at the hind end of the gill region. As in *Pt. minuta* the number of gills never appears to exceed 40 pairs, but in the majority of individuals the number is very much less than this. The gill gut is not closed blindly behind as in *Pt. minuta*, but passes directly over into the gut of the succeeding genital region.

(2) Post-branchial region.—Genital region: The musculature and the dorsal and ventral vessel stems are essentially related as in the branchial region. The wall of the gut (fig. 23, i.) in this region is usually folded, and lies at a varying distance, usually small, from the longitudinal musculature, and there pass

from its ventro-lateral region to the limiting membrane of the epidermis numerous radial fibres. The trunk colom represented in the branchial region by the spaces situated laterally to the esophagus is here considerably reduced and represented by irregular spaces.

The lateral septa (figs. 22, 23, ls.) are related essentially as in the described Ptychodera species. In the middle of the genital region the septa are of considerable breadth, and pass between the dorsal corner of the gut and the submedian line, which is here situated at about the middle of the internal face of each of the genital wings (fig. 23, ls.). Anteriorly the point of attachment of the septum to the gut gradually passes medianly (fig. 22, ls.) until at the level of the last developing gill pocket it passes over to the skin, to which it is then attached at both ends. In the branchial region the ventral point of attachment of the septum gradually approaches the lateral, which always remains at the submedian line until the two unite. Posteriorly to the middle of the genital region the submedian line gradually passes down from the mid-region of the inner face of the genital wing, and takes a position close to its base, and at the same time the septa are reduced gradually in breadth and finally end with a free edge in the anterior portion of the hepatic region. septa thus separate off from the main trunk colom two dorsal chambers which anteriorly end blindly in the posterior part of the branchial region, while posteriorly they communicate with the main trunk colom by their narrow openings. These dorsal chambers Spengel regards as blind-sac-like outgrowths of the trunk cœlom arising in the anterior part of the hepatic region.

Gonads: The gonads begin in the "genital wings" immediately behind the posterior rim of the collar and continue for a considerable distance into the hepatic region. As distinguished from Pt. minuta, in which the gonads are simple unbranched sacs, they are in this species, in correspondence with the greatly developed "genital wings," much branched, and are here more complexly branched than is usually the case in Pt. clavigera. As in these species, only primary gonads and primary genital pores exist. In

the branchial region, as is characteristic of the genus *Ptychodera*, the gonads only exist laterally to the gill pores, and consist of dorsal and ventral gonad branches which are each again subdivided into lesser lobes: the dorsal usually into two long lobes which occupy the uppermost portion of the cavity of the genital wings, the ventral into a number (3-4) of shorter and smaller lobes.

In the genital region proper, as in Pt. clavigera, each gonad consists of three main branches: (1) a dorsal (figs. 22, 23, dab.) occupying, as in the branchial region, the dorsal part of the cavity of the "genital wings," and divided into two long lobes or in some cases into two long lobes and one short one; (2) a ventro-lateral branch situated on the outer side of the lateral septum, between it and the outer body wall, and also subdivided into two lesser lobes (figs. 22, 23, rgb.); (3) a median lateral branch occupying the cavity of the dorsal chamber of the trunk colom, enclosed by the lateral septum and extending in it above the gut towards the median dorsal line (figs. 22, 23, mg.). This median ventral branch is also subdivided into two. In this species, then, there are in connection with each genital pore at least six lesser In fig. 23, a section from about the middle of the branches. genital region is represented which has passed through one of the genital pores (gap.) filled up by a mass of spermatozoa. specimen from a series of which this section is taken was preserved while in the act of extruding the ripe spermatozoa in the form of whitish filmy masses.

Structure of Gonads: According to Spengel the wall of the gonads consists of three layers—an outer peritoneal layer, a middle limiting membrane and an inner germ layer. The outer peritoneal layer is in my preparations very indistinct, and represented by some small flattened nucleated cells' more or less remote from each other and closely applied to the middle limiting membrane. In the latter there is situated a well marked blood sinus. I have never been able to observe a layer of longitudinal muscles in the wall of the gonads in this species. Also, I have not been able to distinguish the germ layer as a distinct layer, but certain small

rounded cells lying irregularly internal to the limiting membrane in young gonads doubtless belong to it. In the young gonads there is present a distinct central cavity filled up in some cases by a deeply staining homogeneous substance. As in the other species of *Ptychodera*, the gonads contain large numbers of rounded or cubical masses of a peculiar substance which in the sexually immature animals fills up the gonads almost completely. It is found in the young gonads, both male and female, but it persists for a much longer time in the case of the ovaries than in the testes. Even in the mature ovaries traces of it remain, while in the mature testes no trace of it is left. Spengel regards this substance as nutritive in function, and the presence of the abundant deutoplasm in the ova readily accounts for its greater persistence in the ovaries.

The ova of this species are essentially similar to those of Pt. minuta. They average about $\frac{1}{10}$ mm. in diameter, and possess very abundant granular deutoplasm. The germinal vesicle is very large, and contains a spongy nucleoplasm in which there is situated one large nucleolus and several smaller masses. The ripe ovum is invested in a thick egg membrane attached to which there may be a few very small flattened nuclei apparently representing the follicular layer. The heads of the ripe spermatozoa are somewhat ovoid or rounded in form, and the flagellum is very long and slender (fig. 26).

Hepatic region: With the exception of the presence of two ciliated bands in the gut, Spengel's description of this region in Pt. minuta applies generally to that of this species. The liver sacs (fig. 24, hep.) communicate with the gut by narrow transverse clefts bounded by anterior and posterior lips. The wall of the gut is thrown into obliquely transverse folds which are interrupted on the dorsal side by the two longitudinal grooves, while a well marked furrow occupies the median dorsal region of the gut.

The liver sacs are lined by a layer of close-set long narrow cells with long nuclei in a row near their base (fig. 24, ehep.). The cells contain numerous yellowish-brown granules, which also occur less abundantly in the somewhat shorter but otherwise similar

cells lining the gut. The cells of the liver gut possess distinct cilia, and in accurate sections through its lining epithelium which are met but seldom, the outer surfaces of the cells form a sinuous line, and no vacuoles are visible in them, so that, as Spengel remarks, the vesicles he has observed in them are probably the result of the action of reagents. On the outer side of the epithelium of the liver sacs and the gut wall there is a rich system of capillary vessels. As in other species of *Ptychodera*, except *Pt. minuta* and *sarniensis*, two ciliated grooves are present in this species, and they show the usual structure. Each consists of a special ciliated band of long columnar cells sunk below the level of the gut epithelium and bounded on its dorsal or median side by a projecting conical mass composed of elongated cells, the "covering pad."

The ciliated grooves extend for a considerable distance into the genital region proper, and they are there situated somewhat below the points of attachment of the lateral septa to the gut wall, and are separated from the epidermis by the thick layer of longitudinal musculature. Posteriorly, about the middle of the hepatic region where the liver sacs are largest, the genital wings are reduced to mere ridges, overhung by the outwardly extended ends of the liver sacs. In fig. 24, the left half of a section about the middle of the hepatic region is represented, and the ciliated groove (cgr.) is seen to lie just below the outer wall of the reduced genital wing (gw.) and now quite close to the epidermis, being separated from it only by a thin layer of the longitudinal musculature which becomes thinner just at this point. The genital wings can be traced as mere elevations of the epidermis below which the ciliated grooves are situated to near the posterior end of the hepatic region.

Hind Body: The course of the ciliated grooves behind the hepatic region is marked externally by two longitudinal epidermal stripes which, commencing laterally to the posterior small liver sacs, extend over the anterior two-thirds of the hind-body region. The hind body can thus be divided into an anterior abdominal region characterised by the presence of the two epidermal stripes

and the underlying ciliated grooves, and into a posterior caudal region.

In the abdominal region the ciliated grooves may be situated directly under the epidermal stripes or may be remote from it by a short distance, but always the portion of the longitudinal musculature between them is somewhat thinner than elsewhere. The cells of the epidermal stripes usually stain darker, and are somewhat lower than the adjacent cells, and in one case where the groove was situated immediately under the epidermal stripe there existed what appeared to be a special differentiation of the cells of the latter in the form of a small mass of cells with rounded nuclei and distinct nucleoli. In other respects the abdominal region of this species closely agrees with that of Pt. minuta. wall of the gut in this region (fig. 25, i.) is thrown into numerous somewhat irregular transverse folds, and it becomes further removed from the longitudinal musculature so that the celom is very much more distinct than in the liver region and, as there, divided into two distinct halves only, however, in the anterior part of the The band-like cellular mass which Spengel has found in Pt. minuta and sarniensis in connection with the dorsal vessel and lying along the dorsal median line of the gut also exists in this species. It has here the form of a low band of cells of varying height lying as in the described species between the two lamellae of the boundary membrane of the gut and not limited laterally. Between the dorsal vessel and the cellular mass there passes a short mesentery, and by the separation of this into two lamellae the cavity of the dorsal vessel comes into direct connection with the cellular mass. At these points the cells of the mass are not closely packed, and have a branching character exactly like the endothelial cells of the dorsal vessel with which they seem to be continuous.

The caudal region of the hind body is essentially similar to that of *Pt. minuta*. As in the posterior part of the abdominal region, the two halves of the colom here stand in connection below the dorsal vessel. On the ventral side the ventral vessel disappears as a distinct structure at the beginning of this region, while the

dorsal vessel retains its lumen to near the posterior end of the body.

As Spengel has found in *Pt. minuta*, the place of the ventral vessel is taken by the keel-shaped process of the gut epithelium. As in *Pt. minuta*, a very delicate musculature is found on the gut in this region, and as in that species a distinct sphincter derived from the circular musculature exists round the anus.

Vascular System.—Dorsal vessel: In the post-branchial region the dorsal vessel is related essentially as in Pt. minuta. It can be traced as a vessel with a distinct lumen to near the posterior end of the body; it appears, however, to end as a solid structure at its extreme posterior end just as in Pt. minuta. In the branchial region the dorsal vessel may occupy the whole of the mesentery or only its dorsal portion. Between the perihæmal spaces the dorsal vessel usually occupies the whole extent of the partition wall, but just as in Pt. minuta it may only occupy the dorsal half of it, and the ventral part of the wall may then either persist as a low wall of partition below the vessel, or it may disappear entirely and place the two perihæmal spaces in communication.

At the anterior end of the perihæmal spaces, the dorsal vessel opens, as in the described species, into a lacuna (figs. 11, 12, 14, cl.) between the organs in the proboscis neck and which stands in connection with the central blood space of the proboscis by a narrow cleft. From the lacuna there passes away dorsally a vessel (fig. 10, oav.), the afferent skin vessel, which Spengel regards as carrying blood to the capillary net of the proboscis. Where two proboscis canals are present it passes between them (fig. 10); where there is only one canal, along one side of that. It then passes into the limiting membrane and runs in that anteriorly for a short distance, finally dividing as in the described species into two branches which divide up again to form the capillary net of the proboscis.

Proboscis glomerulus: The glomerulus of this species essentially resembles that of *Pt. minuta*. It covers the anterior ends of the

heart bladder and the proboscis gut, but just behind the anterior ends of these it becomes limited to two lateral masses lying on the heart bladder and the notochord, and to a small median portion on the dorsal side of the heart bladder. This median portion which stands in connection with the lateral portions by vessels on the walls of the heart bladder posteriorly gives rise to two or three large longitudinal vessels which finally unite to form one main vessel (fig. 3, mgl.) which passes obliquely backwards and upwards along the dorsal edge of the heart bladder (fig. 5, esv.), and comes into connection with the capillary net of the proboscis. Ventrally, also, the lateral masses stand in connection with the capillary net by a network of vessels in the ventral septum of the proboscis. According to Spengel, these vessels, dorsal and ventral, probably act as the efferent skin vessels, i.e., they probably convey the blood from the capillary net of the proboscis to the glomerulus. The glomerulus vessels themselves are similar in their relations to those of Pt. minuta. As Spengel has shown, these vessels represent a honeycomb-like system. As in that species corresponding to the floor of the honeycomb there is a sinus on the lateral walls of the heart bladder which communicates with the central blood space by narrow clefts. From the sinus there radiate outwards vessels which, in longitudinal vertical sections, are readily seen to be connected in a net-like manner, and at the periphery of the glomerulus they give rise to a network of much larger vessels (figs. 3, 4, 5, 6, ql.). The latter opens into a longitudinal vessel occupying the ventral corner of each half of the glomerulus, and which in this species can be traced to near the anterior end of each half of the glomerulus (figs. 3, 4, 5, epv.). These vessels Spengel terms the efferent proboscis vessels, and according to him they arise at the posterior end of the glomerulus. In this species they certainly become distinct at the posterior end of the glomerulus (fig. 8, epr.), but they can be followed up from here as distinct vessels lying in the ventral corner of each half of the glomerulus to near its anterior end.

From a comparative study of the glomerulus, and from its histology, Spengel is led to regard the glomerulus as a system of

infoldings of the splanchnic epithelium, the spaces between these infoldings being filled with blood and representing the glomerulus vessels. For this view speaks the arrangement of the nuclei which occur more or less regularly along the course of the vessels. Round the nuclei an oval non-staining cell body can frequently be recognised, and they can in some places, as Spengel has observed, pass directly over into the splanchnic epithelium.

Efferent Proboscis Vessels: The efferent proboscis vessels after they leave the glomerulus are essentially similar in their course and disposition to those of Pt. minuta. However, as Koehler* has found in Pt. sarniensis, the two efferent proboscis vessels are connected with each other in the proboscis neck by a well-marked vessel (figs. 10, 14, cv.) which passes, in the "chondroid tissue" occupying the space between the anterior portion of the "keel" of the proboscis skeleton and the posterior portion of the "end plate." I have met this connecting vessel not only in transverse series, but also in both vertical and horizontal longitudinal series, and there can, in my opinion, be no doubt as to its existence in this Spengel, however, asserts that the efferent proboscis vessels "never stand in connection with each other," and believes "Koehler has been apparently deceived through the intense colouration with carmine of certain parts of the skeleton which thereby become very similar to the blood fluid."† A series of transverse sections through an individual of the species under consideration, whose vessels were richly filled with coagulated blood, leaves me in no doubt on the matter, and the appearance presented by the vessel as seen in two adjacent sections is represented in fig. 10 (cv.). The specimen was stained with cochineal in 70 % alcohol with the result that the coagulated blood stained a much deeper tint than the proboscis skeleton, allowing the two to be very easily distinguished, and moreover the coagulated blood in the connecting vessel could be distinctly seen to pass over at both ends into that in the efferent proboscis

^{*} Contribution à l'étude des Entéropneustes – Internat. Monatsschrift f. Anat. u. Histologie, Bd. iii. 1886, p. 174

[†] Loc, cit., p. 633.

vessels. Further, the "keel" and "body" of the proboscis skeleton are at this point quite distinct from each other and separated by the narrow ventral band of "chondroid tissue," and there is certainly no median prolongation anteriorly from the point of fusion of the "body" and "keel" of the proboscis skeleton which takes place just behind the connecting vessel, and which could be mistaken for the vessel in question.

We may then take it as characteristic of *Pt. australiensis*, at least, that the efferent proboscis vessels are united by a connecting vessel passing in the "chondroid tissue" between the "body" and "keel" of the proboscis skeleton.

Further, in this species the capillary net of the proboscis comes directly into connection with the efferent proboscis vessels in the proboscis neck, and indeed anteriorly to the connecting vessel. In sections through the proboscis neck, in the region of the ventral blind sac, vessels are found in the here commencing "chondroid tissue" which, as Spengel has shown, is simply the thickened limiting membrane of this region into which cellular strands derived mainly from the proboscis pockets have penetrated. Some of these vessels enter the efferent proboscis vessels (fig. 9), and they thus serve to place the capillary net of the proboscis directly in connection with the efferent proboscis vessels, while the dorsal and ventral efferent skin vessels, since they return the blood first to the glomerulus, do so indirectly.

Vessels of Collar: The efferent proboscis vessels are continued into the collar, and are related there essentially as in the described species of Ptychodera. They appear on their entrance into the collar as clefts in the limiting membrane on either side of the proboscis skeleton, and have at first a longitudinal direction. Very soon they diverge outwards in a fold of the limiting membrane and finally pass downwards round the mouth cavity in a fold of the limiting membrane of the inner wall of the collar—the circular vessel fold. Their dorsal portions are formed of single vessels (fig. 16, cvc.) which ventro-laterally give rise to two capillary nets (fig. 17, cvc'.) which unite in the mid-ventral line of the anterior part of the collar to form the longitudinal ventral

vessels of the collar, formed by folds of the outer limiting membrane of the peripharyngeal space.

The circular vessels stand in direct connection as in the described species with the capillary net in the outer limiting membrane of the peripharyngeal space, and also anterior to the line of termination of the latter with the capillary vessels below the epithelium immediately surrounding the mouth aperture.

The ventral longitudinal vessels generally consist in this species of two distinct folds which may be secondarily branched (fig. 18, lvc.). The longitudinal vessels may, however, be represented by a single simple or much branched fold, or of three or four distinct folds.

As in the described species the ventral longitudinal vessel folds open at their posterior ends into a lacuna in the collar-trunk septum, into which the circular vessel passing in the septum immediately below the circular nerve ring opens laterally, and from which the ventral longitudinal vessel of the trunk takes its origin.

Capillary System of Collar is related essentially as in the described species. On the dorsal side, the vessels in the limiting membranes of the "roots" placing the outer and inner capillary nets in connection are strongly developed (fig. 15). Ventrally, in the hind half of the collar by the formation of a longitudinal fold of the limiting membrane of the epidermis, a median longitudinal subepidermic vessel (fig. 18, slv.) may in some cases be formed opposite the ventral longitudinal plexus. Posteriorly, the former fuses with one of the folds of the latter, and the capillary net of the inner collar wall is thus brought on the ventral side into direct connection with that of the outer. In some individuals, however, the ventral mesentery may be entirely absent, and no such connection established.

The capillary net surrounding the dorsal cord is in this species strongly developed, and especially on its dorsal side (figs. 15 and 17) well-marked vessels (fig. 17) pass off, from the dorsal side of the dorsal vessel, in the limiting membrane between the perihamal spaces and the collar cord. These laterally pass upwards in the

limiting membrane of the collar cord to form the capillary net on on its dorsal surface, and branches also pass downwards in the boundary membrane laterally to the perihamal spaces and come into direct connection with the capillary net of the inner wall of the collar. Very few vessels leave the ventral side of the dorsal vessel in this species, and the capillary net of the inner wall of the collar thus appears to stand mainly in connection with vessels derived from the dorsal side of the dorsal vessel, in common with the capillary net of the collar cord.

Ventral Vessel of Trunk: The ventral vessel is related exactly as in Pt. minuta. It does not extend so far posteriorly as the dorsal, but disappears at the point of origin of the keel-shaped process of the gut.

Lateral Vessels: The lateral vessels are essentially similar to those of the described species. In the branchial region they occupy the usual position just below the submedian lines (fig. 20, llv.). Then when the lateral septa appear, they run in these (fig. 22, llv.). Towards the ends of the septa the vessels gradually pass downwards towards the point of attachment of the septa to the gut, and then behind the termination of the septa they continue as free vessels lying in the interior of the now greatly reduced genital wings (fig. 24, llv.) and which finally about the middle of the hepatic region pass over into the gut capillaries. In this part of their course the vessels possess, as in the other species, circular muscle fibres in their walls, and also from the latter there radiate outwards fine fibres (fig. 24, llv.), probably functioning as expansors.

Gill Vessels: My observations on this very complicated set of vessels confirm, so far as they go, those of Spengel. The afferent gill vessels arising from the dorsal vessel vary according as the dorsal vessel occupies the whole of the mesentery or only its dorsal half. In the former case the afferent vessels pass off directly from the ventral side of the dorsal vessel; in the latter they diverge outwards and downwards from its ventral side, the mesentery being apparently absent at this point. The afferent

vessels arise from the dorsal vessel opposite the gill tongues. Each, according to Spengel, divides soon after its origin into two vessels—one of which passes into the tongue and divides again to form the two vessels running along the tongue prongs, the other passes to the gill septum in front of the tongue which received the first vessel, and probably becomes continuous with the septal vessel. I have not been able to make out from my preparations the above described connections to my complete satisfaction. Each of the afferent vessels seems to stand in connection with a vessel lying just above the line of attachment of the gill tongue, and this vessel extends on to the dorsal edge of the succeeding septum, and is situated just above the dorsal end of the gill skeleton. Through this vessel there is doubtless established the connection between the blood flowing in the gill tongues and that in the septa.

The vessels in the tongue are exactly as described by Spengel. In transverse sections through the branchial region the capillary net in the tongue situated immediately below the peritoneal covering of the tongue cavity (fig. 20, cnt.) can be distinctly seen, and especially in horizontal sections the larger vessels of the net, viz., the large vessel situated immediately under the epithelium of the "tongue back" and the two smaller vessels along the inner side of each tongue prong (fig. 21, tp.) can be distinctly recognised (fig. 21). In each of the gill septa the septal vessels (figs. 20, 21, vgs.) can readily be made out running just external to the septal prong (spr.). At their ventral ends the septal vessels stand in connection with the "limiting vessel" below the boundary cushion between the branchial canal and the esophagus, and this "limiting vessel" stands in the usual connection with the capillary net on the wall of the esophagus.

Though none of my preparations show a distinct capillary net in the gill septa, in some I have observed small branches passing from the septal vessel, and these probably represent part of the capillary net Spengel has found so richly developed in the gill septa of *Pt. sarniensis*.

EXPLANATION OF PLATES.

Reference Letters.

ag. Projections of the anterior ends of the gill rows into the throat. av. Afferent skin vessels of proboscis. bps. Body of proboscis skeleton. bv. Limiting vessel of branchial region. cbs. Central blood space of proboscis. ccp. Prolongations of collar cœlom into the proboscis neck. cfw. Circular musculature of outer wall of anterior rim of collar, cyr. Ciliated groove. ch. "Chondroid tissue." ciw. Capillary net of inner wall of collar. cl. Cleft into which dorsal vessel opens. cm. Circular musculature of proboscis. cnc. Collar cord. cnp. Capillary net of proboscis. cnt. Capillary net of gill tongue. ca. Collar colom. ca. Parts of collar colom into which collar pores open. cp. Covering pad. ct. Inner layer of connective tissue of proboscis on the splanchnic epithelium. cr. Vessel connecting the two efferent proboscis vessels in the proboscis neck. evc. Dorsal portion of circular vessel fold of collar. eve'. Ventral plexus of circular vessel fold of collar. dgb. Dorsal gonad branches. div. Notochord. dn. Dorsal nerve stem of trunk. dp. Dorsal proboscis pockets, Fibres of dorso-ventral muscle plate. dv. Dorsal vessel of trunk. Epithelium of liver sac. ep. Epidermis. epb. Epibranchial strand. "End plate" of proboscis skeleton. epth. Epithelium of throat. Efferent proboscis vessels. esr. Efferent proboscis skin vessels on front edge of heart bladder. g. Gonads. gap. Genital aperture. gc. Gill cleft. gg. Branchial canal. gl. Glomerulus. glm. Muscle fibres of glomerulus. ap, Gill pore. as. Gill septum. at. Gill tongue. atb. Epithelium of gill tongue back. gtc. Gill tongue cavity. gw. Genital wings. h. Heart bladder. hep Cavity of liver sac. i. Intestine. ies. Intermediate epithelial stripe of gill tongue. ifw. Musculature of fore wall of anterior rim of collar. ilm. Inner longitudinal musculature of collar. ilm'. Portion of same passing to the proboscis base. icw. Infolding of ventral wall of heart bladder. ivw'. Anterior projection of the same into the cavity of heart bladder. kps. "Keel" of proboscis skeleton. lb. Anterior horn of blind sac lumen. lbs. Ventral blind sac of notochord. lc. Limiting cushions between branchial canal and œsophagus. Ifw. Longitudinal musculature of outer wall of anterior rim of collar. Uv. Lateral longitudinal vessel. Im. Longitudinal musculature of proboscis. Imt. Longitudinal musculature of trunk. lmv. Longitudinal musculature of ventral wall of perihæmal spaces. lps. "Legs" of proboscis skeleton. lps'. "Nuclei" of proboscis skeleton. ls. Lateral septa of trunk. lvc. Longitudinal ventral vessel folds of inner wall of collar. mg. Median gonad branches. mgl Vessel passing from the median dorsal portion of the glomerulus. nf. Nerve fibre layer. Nerve fibre ring of proboscis neck. oav. Dorsal prolongation of the blood cleft in proboscis neck to form the afferent skin vessels. a. Esophagus. ol. Opening of lumen of notochord into the throat. p. Proboscis pore. pbc. Proboscis cavity. pc. Proboscis canal. phs. Perihæmal spaces. pps. Peripharyngeal space. rf. Radial fibres passing between the outer and fore walls of anterior rim of collar. rt. "Roots." scn. Subepidermic capillary net of collar. slv. Median longitudinal vessel of the subepidermic capillary net in the posterior ventral part of the collar. sn. "Synaptikel." sp. Splanchnic epithelium of proboscis cœlom. spr. Septal prong of gill skeleton. thh. Throat or mouth cavity. tpr. Tongue prongs of gill skeleton. trc. Trunk cœlom. vcs. Ventral collar septum. vgb. Ventral gonad branches. vgs. Septal vessel. vn. Ventral nerve stem. vp. Ventral proboscis pockets. rps. Ventral septum of proboscis. vv. Ventral vessel of trunk. vv. Ventral wall of heart bladder.

PTYCHODERA AUSTRALIENSIS.

Plate 1.

- Fig. 1.—Enlarged dorsal view of a small preserved specimen.
- Fig. 2.—Dorsal view of a portion of the abdominal region showing the two epidermal stripes. (\times 3.)
- Fig. 3.—Central part of a transverse section of the proboscis passing through the anterior free projection of the infolded ventral wall of the heart bladder. Zeiss A., oc. 1., cam. luc.
- Fig. 4.—Transverse section slightly posterior to fig. 3, but from another series especially showing the infolding of the ventral heart bladder wall. Zeiss A., oc. 1., cam. luc.

Plate II.

- Fig. 5.—Transverse section passing through about the middle region of the proboscis organs. Zeiss A., oc. 1., cam. luc.
- Fig. 6.—Portion of glomerulus in transverse section. Zeiss D., oc. 1., cam. luc.
- Fig. 7.—Transverse section of the splanchnic epithelium on the notochord.

 Zeiss D., oc. 1., cam. luc.

Plate III.

- Fig. 8.—Transverse section through the beginning of the proboscis neck. Zeiss A., oc. 1., cam. luc.
- Fig. 9.—Transverse section of proboscis neck posterior to fig. 8 and passing through the ventral blind sac of the notochord. Zeiss A., oc. 1., cam. luc.
- Fig. 10.—Transverse section of proboscis neck passing through the proboscis canals and the connecting vessel between the efferent proboscis vessels. Zeiss A., oc. 2., cam. luc.

Plate IV.

- Fig. 11.—Transverse section of proboscis neck of an individual with two proboscis pores. Zeiss A., oc. 2., cam. luc.
- Fig. 12.—Similar section of an individual with a single median proboscis pore. Zeiss A., oc. 2., cam. luc.
- Fig. 13.—Dorsal view of proboscis skeleton. (x about 30).
- Fig. 14.—Vertical longitudinal section (nearly median) through the point of union of the proboscis neck with the collar. Zeiss A (without lowest lens), oc. 1., cam. luc.

Plate v.

- Fig. 15.—Vertical longitudinal section through the dorsal region of collar Zeiss A., oc. 1., cam. luc.
- Fig. 16.—Dorsal median portion of a transverse section through the anterior region of collar. Zeiss A., oc. 1., cam. luc.

Plate VI.

- Fig. 17.—Transverse section through the collar passing through the opening of the notochord into the throat and through the circular vessel fold. Zeiss A (without lowest lens), oc. 1., cam. luc.
- Fig. 18.—Transverse section through the posterior region of collar. Zeiss A (without lowest lens), oc. 1., cam. luc.

Plate VII.

- Fig. 19.—Transverse section of ventral part of collar slightly posterior to fig. 18, showing the ventral septum. Zeiss A., oc. 1., cam luc.
- Fig. 20.—Transverse section (slightly oblique) through the branchial region of a sexually immature individual. Zeiss A (without lowest lens), oc. 1., cam. luc.
- Fig. 21.—Section of a single gill from a vertical longitudinal series. Zeiss C., oc. 1., cam. luc.
- Fig. 22.—Transverse section of a sexually immature individual, just behind the branchial region. Zeiss A (without lowest lens), oc. 1., cam. luc.

Plate VIII.

- Fig. 23.—Transverse section through the genital region of a sexually mature male, passing through a genital pore. Zeiss A (without lowest lens), oc. 1., cam. luc.
- Fig. 24.—Transverse section through the hepatic region. Zeiss A., oc. 1., cam. luc.
- Fig. 25.—Transverse section through the abdominal region showing the two ciliated bands. Zeiss A., oc. 1., cam. luc.
- Fig. 26.—Ripe spermatozoa. Zeiss D., oc. 4., cam. luc.